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Report No. IITRI-L6018-4

SUSCEPTIBILITY TO INFECTION IN IRRADIATED ANIMALS

Annual Report

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and
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U.S. Army Medical Research and Development Command
Office of the Surgeon General
Washington, D.C. 20315

Contract No. DA-49-193-MD-2630

Contractor: IIT Research Institute
Chicago, Illinois 60616

FOREWORD

On July 1, 1964, a research program was initiated by IIT Research Institute for the U.S. Army Medical Research and Development Command, Office of the Surgeon General, Washington, D.C. 20315, to study susceptibility to infection in irradiated animals. This is Report No. IITRI-L6018-4 (Annual Report) on IITRI Project L6018, Contract No. DA-49-193-MD-2630, entitled "Susceptibility to Infection in Irradiated Animals."

This report covers the period from July 1, 1964, to June 30, 196 and describes the research conducted to study the effect on mice of exposure to various levels of gamma radiation and subsequent infection with Bacillus anthracis.

In conducting the research described in this report, the investigators adhered to the "Principles of Laboratory Care" as established by the National Society for Medical Research.

Merl Kardatzke participated in the design of the experiments and performed the statistical analysis of the data. The irradiation procedures were conducted by Wanda Bahmet and Judy Wurmel. Participating in the technical performance of the program at various times during the year were Herbert Logan, Sylvia Girtz, Lynne Rutzky, and Catherine Rusnock.

The data contained in this report are recorded in Logbooks C15141, C15364, C15426, C15501, C15664, C15839, and C15859.

The information in this report has not been cleared for release to the general public.

The findings in this report are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

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SUMMARY

SUSCEPTIBILITY TO INFECTION IN IRRADIATED ANIMALS

This program was conducted in order to investigate the effects of irradiation on the susceptibility of animals to infection. Virulent and avirulent strains of Bacillus anthracis were used as the infectious agents, and Swiss albino mice were used as the test animals. Block design experiments were conducted to determine how the mortality of the mice was affected by varying independently the radiation and the bacterial challenge. The radiation varied from 100 to 900 rads of Co^{60} gamma rays, and the infectious challenge varied from 0 mortality to approximately an LD_{50}. Mice were exposed to radiation 1, 3, or 7 days before infectious challenge. A pronounced combined effect occurred; i.e., the mortality was significantly higher than could be expected by the independent action of radiation and infection. The effect of the anthrax and radiation doses on the combined effect was linear with increasing dose. At low levels of radiation bacterial challenges near the LD_{50} were necessary to produce a combined effect, while at high levels of radiation only a few organisms sufficed to produce a pronounced combined effect. The time interval between radiation exposure and infectious challenge was significant; the combined effect increased from the 1-day interval to the 7-day interval. A combined effect significant at the 95% probability level can be demonstrated with a radiation dose of approximately 200 rads. Hence, prior exposure to even low levels of Co^{60} gamma radiation significantly increased the mortality of mice infected with B. anthracis.

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SUSCEPTIBILITY TO INFECTION IN IRRADIATED ANIMALS

I. INTRODUCTION

It has been well documented in the literature that exposure to ionizing radiation can result in increased susceptibility to experimentally induced bacterial infection. In most of these studies, increased susceptibility has been demonstrated as a decrease in the number of organisms required to produce an LD_{50} or as increased mortality produced by specified bacterial challenge levels. The objective of this program is to more rigorously define the degree of interaction between irradiation and challenge with various microbial pathogens. These studies should also give some insight into the problem of how to determine the minimal radiation dose that increases susceptibility to infection.

Experiments were conducted to obtain information concerning the magnitude of the combined effect of radiation dose and bacterial challenge. Block design experiments were carried out with independently varied levels of radiation and infectious challenge. Bacillus anthracis was selected as the infectious organism because it served as a model for a bacterial pathogen and had the advantage of being available in virulent and avirulent strains. We could therefore explore two systems in which widely different numbers of organisms were required to reach the same end point.

II. METHODS

Male Swiss albino Ha/ICR strain mice (A. R. Schmidt Co., Madison, Wis.) were used. All the mice used in an experiment were received on the same day. After 3 to 4 days, the mice were divided into groups on the basis of weight: 18 to 21 g and 21 to 24 g. Each group contained 1080 mice, which were housed in groups of 10 mice per cage. Each weight group was further divided into 3 groups, each containing 360 mice, by using a table of random numbers for the selection of cages. The mice were further randomized by separating the 10 mice in each cage, so that the final cages contained no more than 2 mice from any one original cage.

The mice were exposed to radiation in the high-level Co^{60} gamma facility at IITRI Research Institute. The mice were placed in a specially designed plastic exposure cage consisting of 100 cells for individual housing of mice (Figure 1). To obtain a dose rate that did not vary by more than 5% over the entire exposure area, three Co^{60} sources were positioned above the cage. The sources were held rigidly in place so that the source geometry could be readily reproduced. To obtain accurate radiation dosimetry, capsules of ferrous sulfate were placed in each mouse cell, and

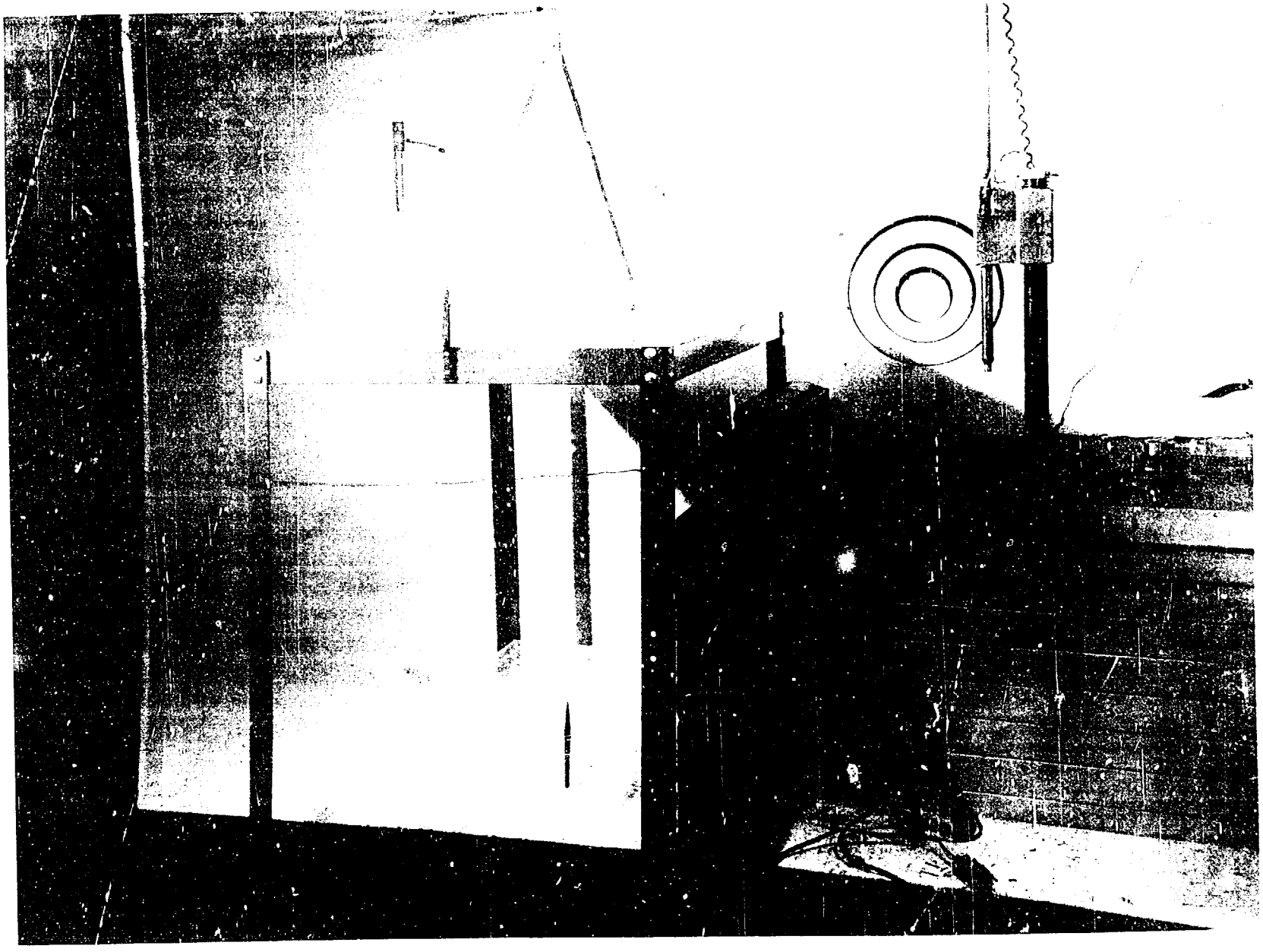

Figure 1

IRRADIATION OF MICE IN THE Co^{60} GAMMA FACILITY AT IIT RESEARCH INSTITUTE
(a) Holders for radiation sources. (b) Cage assembly for mice.

the cage was exposed to the Co^{60} sources for approximately 16 hr. The dose rate within 74 of the 100 cells was 1390 ± 65 rads/hr. Only these cells were used in the animal exposures.

For irradiation, the cage containing the mice was placed in the hot cell and the three sources were placed in position at 1-min intervals. At the conclusion of the irradiation the sources were removed in the same order at 1-min intervals. Following irradiation, the mice were housed in standard shoebox-type stainless steel cages and placed in a holding room.

The cultures were obtained from the U.S. Army Biological Laboratories, Fort Detrick, Maryland. They were designated 32S for the virulent strain and 32R for the avirulent strain. Both these strains are variants of the Vollum B strain.[1] Sufficient quantities of spores were obtained by growing the organisms in intussuscepted cellulose dialysis tubing and aerating the culture with oxygen periodically during the incubation period.[2] After a 4-day incubation period, the cultures were heat-shocked at 65°C for 30 min, quickly frozen, and stored at dry ice temperature.

A group of high-weight mice and a group of low-weight mice were exposed to radiation 1, 3, or 7 days before inoculation. In the experiments utilizing the virulent strain of B. anthracis, 32S, groups of 60 mice were exposed to radiation doses of 100, 300, 500, 700, or 900 rads. These doses were chosen so as to include sublethal through LD_{50} exposures. In later experiments, utilizing the avirulent strain of B. anthracis, 32R, the mice were exposed to 100, 300, 400, 500, or 700 rads because a high mortality was obtained in the mice exposed to 900 rads.

All the mice constituting one experiment were inoculated on the same day. Ten high-weight and ten low-weight mice exposed at each radiation dose, from each of the three time interval groups, were inoculated with one of five different concentrations of B. anthracis. The highest concentration of organisms chosen was determined to produce approximately a 50% mortality. The mice were inoculated intraperitoneally with 0.5 ml of gelatin phosphate containing the desired concentration of organisms. The control groups were mice that had not been exposed to gamma radiation or B. anthracis, mice exposed only to gamma radiation, and mice exposed only to B. anthracis. The mice were observed for 30 days after radiation exposure, and the mortality was recorded daily.

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[1] Fernelius, A. L., DeArmon, I. A., Klein, F., and Lincoln, R. E., Comparison of graded and quantal virulence tests for Bacillus anthracis spores. J. Bacteriol. 79, 594-600 (1960).

[2] Schneider, M. D., Grey, N., and Anellis, A., Sporulation of Clostridium botulinum types A, B, and E, Clostridium perfringen and putrefactine anaerobe 3679 in dialysis sacs. J. Bacteriol. 85, 126-133 (1963).

A virulent V1b-189, strain of B. anthracis, was obtained from Dr. Martha Ward, U.S. Army Medical Unit, Fort Detrick, Fredrick, Maryland. Small block design experiments utilizing the virulent V1b-189 B. anthracis strain were conducted to determine the effect of utilizing other B. anthracis cultures in the combined radiation and anthrax experiments. Im these experiments the mice were exposed to 100, 300, or 500 rads of gamma radiation and 3 days later were challenged with three different levels of infectious organisms.

III. RESULTS, DISCUSSION, AND CONCLUSIONS

The results of duplicate experiments with the virulent strain of B. anthracis were grouped and are presented in Table 1, and similar data on the avirulent strain of B. anthracis are presented in Table 2.

To assess the degree of combined effect, a null hypothesis was advanced that considered the radiation and the bacterial challenge to be acting independently; that is, the probability of survival at each combined exposure point would be the product of the independent probabilities of survival. These products are the calculated percent survivals. Tables 3 and 4 present the calculated and the experimental percent survivals for the virulent and avirulent groups, respectively; the data for the three time periods of exposure to radiation were grouped.

The difference between the number of actual survivors and those calculated from the no-interaction hypothesis represents the excess deaths due to the combined effect. At high dosage combinations the survivors expected are few, and thus there is a finite limit on the possible number of excess deaths. This limit reduces the significance of the number of excess deaths occurring at each combined exposure point. By converting the data to percentage of excess deaths, however, the magnitude of the combined can be clearly seen (Table 5).

The results of both of the experiments were similar, although there was more scatter in the data from the avirulent anthrax experiment. A three-dimensional representation of the data in Table 5 is presented in Figures 2 and 3. The effect of increasing each component and a sharp rise at high levels of radiation and infection can be seen. The magnitude of the combined effect in the avirulent anthrax experiment at high dose combinations is less than that in the virulent anthrax experiment.

Table 1

MORTALITY OF MICE EXPOSED TO RADIATION AND SUBSEQUENTLY INOCULATED WITH VIRULENT *B. ANTHRACIS* STRAIN 32S

Radiation Dose, rads	Mortality, deaths/total Number of Organisms 0	3	15	30	135	270
Mice exposed to radiation 1 day before inoculation						
0	0/40	1/40	4/40	9/40	15/40	18/40
100	0/40	1/40	3/40	8/40	11/40	23/40
300	1/40	7/40	1/40	10/40	10/40	30/40
500	5/40	7/40	3/40	14/40	17/40	31/40
700	30/40	31/40	33/40	32/40	30/40	37/40
900	38/40	30/40	40/40	39/40	36/40	40/40
Mice exposed to radiation 3 days before inoculation						
0	0/40	1/40	4/40	4/40	14/40	22/40
100	1/40	0/40	6/40	5/40	12/40	29/40
300	1/40	1/40	7/40	8/40	24/40	31/40
500	7/40	13/40	18/40	21/40	33/40	31/40
700	22/40	24/40	35/40	40/40	38/40	40/40
900	40/40	40/40	40/40	40/40	40/40	40/40
Mice exposed to radiation 7 days before inoculation						
1	1/40	0/40	2/40	12/40	5/40	22/40
100	2/40	2/40	3/40	17/40	23/40	19/40
300	4/40	1/40	11/40	23/40	18/40	25/40
500	10/40	7/40	16/40	22/40	23/40	33/40
700	36/40	38/40	38/40	37/40	39/40	40/40
900	40/40	40/40	40/40	40/40	40/40	40/40

Table 2

MORTALITY OF MICE EXPOSED TO RADIATION AND SUBSEQUENTLY INOCULATED WITH AVIRULENT *B. ANTHRACIS* STRAIN 32R

Radiation Dose, rads	Mortality, deaths/total — Number of Organisms					
	0	1,000	5,000	10,000	50,000	100,000
Mice exposed to radiation 1 day before inoculation						
0	0/40	9/40	2/40	5/40	12/40	17/40
100	0/40	1/40	8/40	6/40	11/40	17/40
300	1/40	4/40	8/40	7/40	11/40	16/40
400	1/40	3/40	9/40	7/40	16/40	23/40
500	9/40	11/40	11/40	13/40	16/40	22/40
700	23/40	26/40	26/40	26/40	32/40	36/40
Mice exposed to radiation 3 days before inoculation						
0	1/40	11/40	5/40	8/40	10/40	20/40
100	0/40	5/40	11/40	2/40	11/40	18/40
300	0/40	5/40	4/40	16/40	13/40	23/40
400	2/40	7/40	13/40	13/40	20/40	21/40
500	6/40	15/40	15/40	15/40	19/40	31/40
700	31/40	30/40	36/40	36/40	37/40	38/40
Mice exposed to radiation 7 days before inoculation						
0	0/40	2/40	2/40	7/40	7/40	14/40
100	1/40	2/40	2/40	3/40	6/40	17/40
300	3/40	5/40	6/40	11/40	11/40	19/40
400	5/40	6/40	9/40	12/40	20/40	25/40
500	21/40	20/40	29/40	24/40	29/40	32/40
700	35/40	40/40	38/40	38/40	40/40	39/40

Table 3

SURVIVAL OF MICE EXPOSED TO RADIATION AND INOCULATED 1, 3, OR 7 DAYS LATER WITH VIRULENT B. ANTHRACIS, STRAIN 32S

Radiation Dose, rads	Cumulative Percent Survival — Number of Organisms: 0 Exp	3 Exp	3 Calc	15 Exp	15 Calc	30 Exp	30 Calc	135 Exp	135 Calc	270 Exp	270 Calc
0	99	98		92		79		72		48	
100	97	97	95	90	39	71	77	62	70	41	47
300	95	92	93	84	87	66	75	57	68	28	46
500	82	77	80	69	75	52	65	39	59	21	39
700	27	22	26	12	25	9	21	11	19	2	13
900	2	0	2	0	2	1	2	3	2	0	1

Table 4

SURVIVAL OF MICE EXPOSED TO RADIATION AND INOCULATED 1, 3, OR 7 DAYS LATER WITH AVIRULENT B. ANTHRACIS. STRAIN 32R

	Cumulative Percent Survival											
	Number of Organisms											
Radiation	0	1,000		5,000		10,000		50,000		100,000	
Dose, rads	Exp	Exp	Calc	Exp	Calc	Exp	Calc	Exp	Calc	Exp	Calc
0	99	82		93		83		76		58	
100	99	93	81	83	92	91	82	77	74	57	57
300	97	88	79	85	90	72	80	71	72	52	56
400	93	87	76	74	86	73	77	53	69	43	53
500	70	62	57	54	65	48	58	47	53	29	40
700	26	20	21	17	24	16	22	9	19	6	15

Table 5

PERCENTAGE OF EXCESS DEATHS RESULTING FROM EXPOSURE OF MICE TO RADIATION AND INOCULATION WITH B. ANTHRACIS

Radiation Dose, rads	Percentage[a]									
	Virulent Strain, 32S					Avirulent Strain, 32R				
	Number of Organisms					Number of Organisms				
	3	15	30	135	270	1,000	5,000	10,000	50,000	100,000
100	-3	-1	8	12	12	-15	10	-10	-2	0
300	1	3	12	17	38	-12	5	11	3	7
400						-14	14	5	25	20
500	3	7	19	34	47	- 7	17	17	12	27
700	13	53	56	43	81	4	31	27	54	60

[a] $\frac{\text{Survivors expected, theory - survivors found, experimentally}}{\text{survivors expected, theory}} \times 100.$

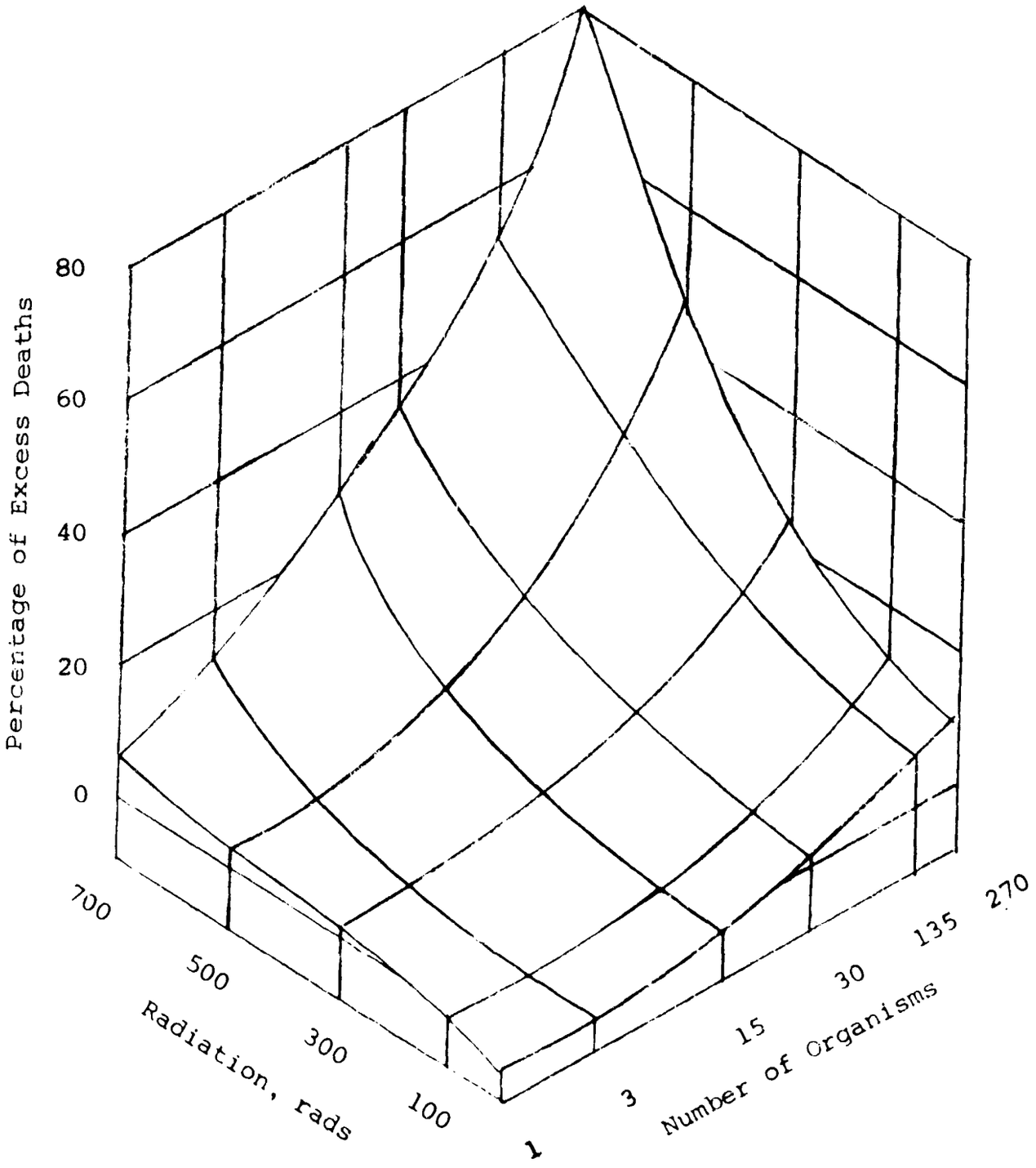

Figure 2

COMBINED EFFECT OF EXPOSURE OF MICE TO RADIATION AND CHALLENGE WITH VIRULENT B. ANTHRACIS, STRAIN 32S

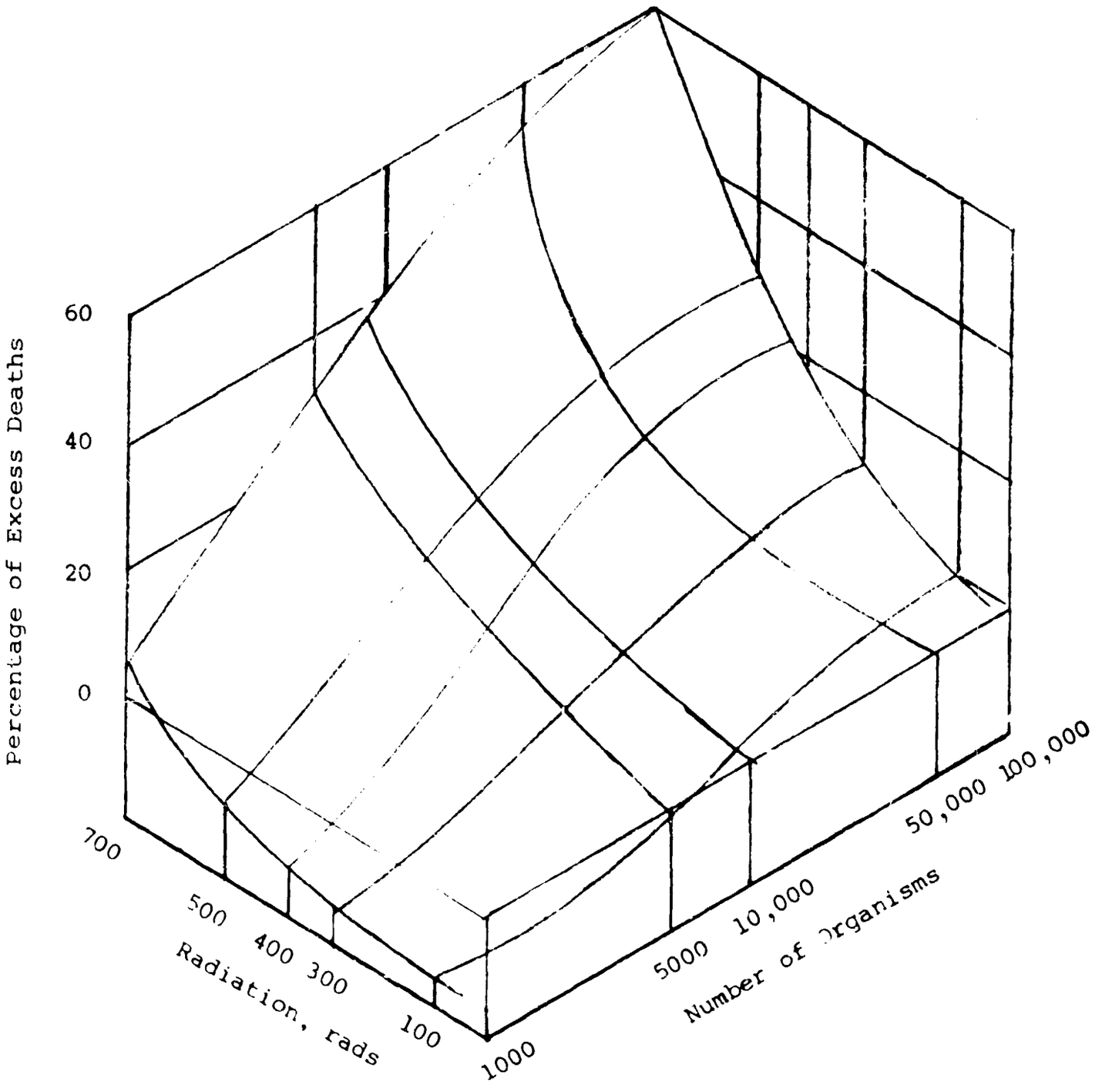

Figure 3

COMBINED EFFECT OF EXPOSURE OF MICE TO RADIATION AND CHALLENGE WITH AVIRULENT B. ANTHRACIS, STRAIN 32S

These experiments represent a summation of a large number of individual data points in which dose of radiation, number of challenge organisms, time interval between irradiation and bacterial challenge, weight range, and duplicate experiments are all independent variables. A statistical analysis on the data was performed on the IBM 7094 computer using an analysis of variance routine obtained from the Biomedical Computer Programs, University of California. A correction was found to be necessary to enable the program to handle the specified maximum number of independent variables.

A summary of the analysis of variance is presented in Tables 6, and Tables 7 and 8 present a detailed breakdown of the main effects. Table 9 presents a detailed breakdown of two-way interactions, and Table 10 presents a breakdown of selected three-way interactions. The selection of the three-way interactions was necessary because of the size of the computer program. The three-way interactions chosen were those most likely to be relevant for testing two-way interactions.

The difference between the virulent anthrax experiments was significant. However, each experiment contained all experimental points, so this difference does not affect the conclusions. Anthrax dose significantly affected the degree of combined effect in a linear fashion with increasing dose level. Radiation also significantly affected the degree of combined effect linearly with increasing dose but also contained some higher-order component. The three-dimensional representation (Figure 2) gives some indication of this at higher radiation exposures. The day effect, or the time interval between radiation exposure and infectious challenge, was significant. The effect increased from the 1-day interval to the 7-day interval. This is illustrated in Figure 4. The mortalities shown are those of all anthrax challenge groups at each radiation level.

The results of the statistical analysis of the data in the avirulent anthrax experiments were similar to those of the virulent experiments with the exception that no higher-order radiation term was found. Also, a significant two-way interaction was found and was attributed mainly to an interaction between the time interval between irradiation and challenge and radiation dose. This phenomenon is illustrated in Figure 5, which shows that the day effect was much more pronounced at higher radiation exposures.

Hence we can conclude that the combined effect demonstrated is a function of radiation dose and level of bacterial challenge. At low levels of radiation, bacterial challenges near the LD_{50} were necessary to demonstrate a combined effect. According to a t test performed on all the animals from all the virulent challenges grouped together, the combined effect was significant at the 95% probability level at a radiation dose of roughly 200 rads. If only exposures to

Table 6

ANALYSIS OF VARIANCE ON ANTHRAX AND RADIATION COMBINED EFFECTS EXPERIMENTS

Effect	Degrees of Freedom	Virulent Anthrax, 32S				Avirulent Anthrax, 32R			
		Sums of Squares	Mean Square	Variance Ratio	P[a]	Sums of Squares	Mean Square	Variance Ratio	P[a]
Mean	1	1.72705	1.7270	30.412	<0.01	0.23533	0.2353	7.418	<0.01
Main effects	14	7.18693	0.5134	9.041	<0.01	6.91481	0.4939	15.572	<0.01
2-way interactions	25	2.39589	0.0958	1.687	N. S.	5.02239	0.2009	6.334	<0.01
3-way interactions	18	1.40407	0.0780	1.374	N. S.	0.74006	0.0411	1.296	N. S.
Residual error	374	21.23788	0.0567			11.86244	0.0317		
Total	432	33.95183				24.77503			

[a]P = probability. N. S. = not significant.

Table 7

DETAILED BREAKDOWN OF MAIN EFFECTS IN THE VIRULENT ANTHRAX AND RADIATION COMBINED EFFECTS EXPERIMENT

Effect	Degrees of Freedom	Sums of Squares	Mean Square	Variance Ratio	P
Weight	1	0.00772	0.00772	0.135	N.S.
Experiment	1	0.68454	0.68454	12.055	<0.01
Day, linear	1	1.07227			
Day, quadratic	1	0.06534			
	2	1.13761	1.13761	20.033	<0.01
Anthrax, 1st and 2nd components[a]	2	1.71099	0.85550	15.065	<0.01
Anthrax, other components	3	0.02024	0.00675	0.119	N.S.
	5	1.73123			
Radiation, 1st and 2nd components[a]	2	2.48770	1.24335	21.895	<0.01
Radiation, other components	3	1.13811	0.37937	6.681	<0.01
	5	3.62581			
Total	14	7.18693			

[a]The anthrax and radiation 1st and 2nd components were, respectively, the dose-no dose and the linear components.

Table 8

DETAILED BREAKDOWN OF MAIN EFFECTS IN THE VIRULENT ANTHRAX AND RADIATION COMBINED EFFECTS EXPERIMENT

Effect	Degrees of Freedom	Sums of Squares	Mean Square	Variance Ratio	P
Weight	1	0.00540	0.00540	0.170	N.S.
Experiment	1	1.40156	1.40156	44.188	<0.01
Day, linear	1	1.33235			
Day, quadratic	1	0.14645			
	2	1.47880	0.73940	23.312	<0.01
Anthrax, 1st and 2nd components	2	1.02666	0.51333	16.184	<0.01
Anthrax, other components	3	0.08275	0.02758	0.869	N.S.
	5	1.10941			
Radiation, 1st and 2nd components	2	2.82343	1.41172	44.087	<0.01
Radiation, other components	3	0.09623	0.03208	1.011	N.S.
	5	2.91966			
Total	14	6.91481			

Table 9

DETAILED BREAKDOWN OF TWO-WAY INTERACTIONS IN THE ANTHRAX AND RADIATION COMBINED EFFECTS EXPERIMENTS

Effect[a]	Degrees of Freedom	Virulent Anthrax, 32S				Avirulent Anthrax, 32R			
		Sums of Squares	Mean Square	Variance Ratio	P	Sums of Squares	Mean Square	Variance Ratio	P
W X E	1	0.01105	0.01105	0.195	N.S.	0.00019	0.00019	0.006	N.S.
W X D	2	0.35798	0.17899	3.152	N.S.	0.46033	0.23016	7.256	<0.01
E X D	2	0.03663	0.01831	0.322	N.S.	0.12834	0.06417	2.023	N.S.
W X A	2	0.03497	0.01748	0.308	N.S.	0.09233	0.04616	1.455	N.S.
W X R	2	0.01167	0.00584	0.097	N.S.	0.02604	0.01302	0.410	N.S.
E X R	2	0.15008	0.07549	1.329	N.S.	0.67414	0.33707	10.627	<0.01
E X A	2	0.26808	0.13404	2.360	N.S.	0. 47091	0.23546	7.424	<0.01
D X A	4	0.54106	0.13526	2.382	N.S.	0.24757	0.12378	3.903	<0.01
D X R	4	0.31050	0.07762	1.367	N.S.	2.83202	0.70800	22.312	<0.01
A X R	4	0.67295	0.16824	2.963	N.S.	0.09055	0.02264	0.714	N.S.
Total 2-way interaction	25	2.39589				5.02239			

[a] W = weight; E = experiment; D = day; A = anthracis; R = radiation. In the radiation and anthracis, the 1st and 2nd components were the only ones used to test for interactions. These were the dose-no dose and linear components respectively.

Table 10

BREAKDOWN OF THE SELECTED THREE-WAY INTERACTIONS IN THE ANTHRAX AND RADIATION COMBINED EFFECTS EXPERIMENTS

Effect[a]	Degree of Freedom	Virulent Anthrax, 32S				Avirulent Anthrax, 32R			
		Sums of Squares	Mean Square	Variance Ratio	P	Sums of Squares	Mean Square	Variance Ratio	P
W X E X D	2	0.06463	0.03232	0.569	N.S.	0.00856	0.00428	0.135	N.S.
W X E X A	2	0.13210	0.06605	1.163	N.S.	0.07446	0.03723	1.174	N.S.
W X E X R	2	0.17601	0.08800	1.550	N.S.	0.11793	0.05896	1.859	N.S.
W X A X D	2	0.09121	0.04560	0.803	N.S.	0.12126	0.06063	1.912	N.S.
W X R X D	2	0.15694	0.07847	1.382	N.S.	0.00025	0.00012	0.004	N.S.
E X D X A	4	0.40268	0.10067	1.773	N.S.	0.12635	0.03159	0.996	N.S.
E X D X R	4	0.38047	0.09512	1.675	N.S.	0.29128	0.07282	2.296	N.S.
Total 3-way interaction	18	1.40407				0.74007			

[a]The 1st and 2nd components were the only ones used to test for interactions and were the dose-no dose and linear components. The linear day effect was used because of the limitations on the size of the analysis of variance programs.

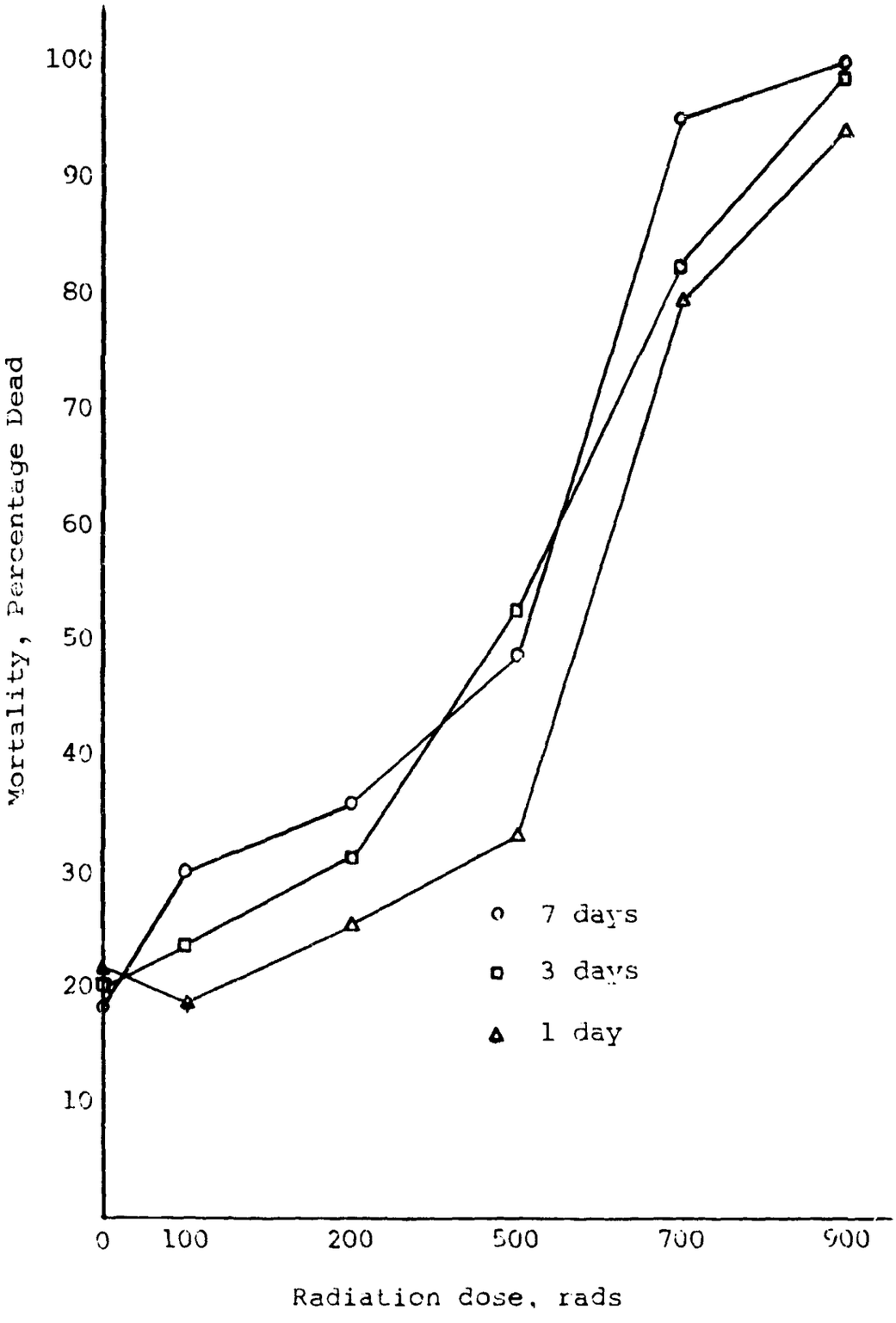

Figure 4

EFFECT OF TIME BETWEEN RADIATION AND BACTERIAL CHALLENGE WITH VIRULENT B. ANTHARCIS, STRAIN 32S

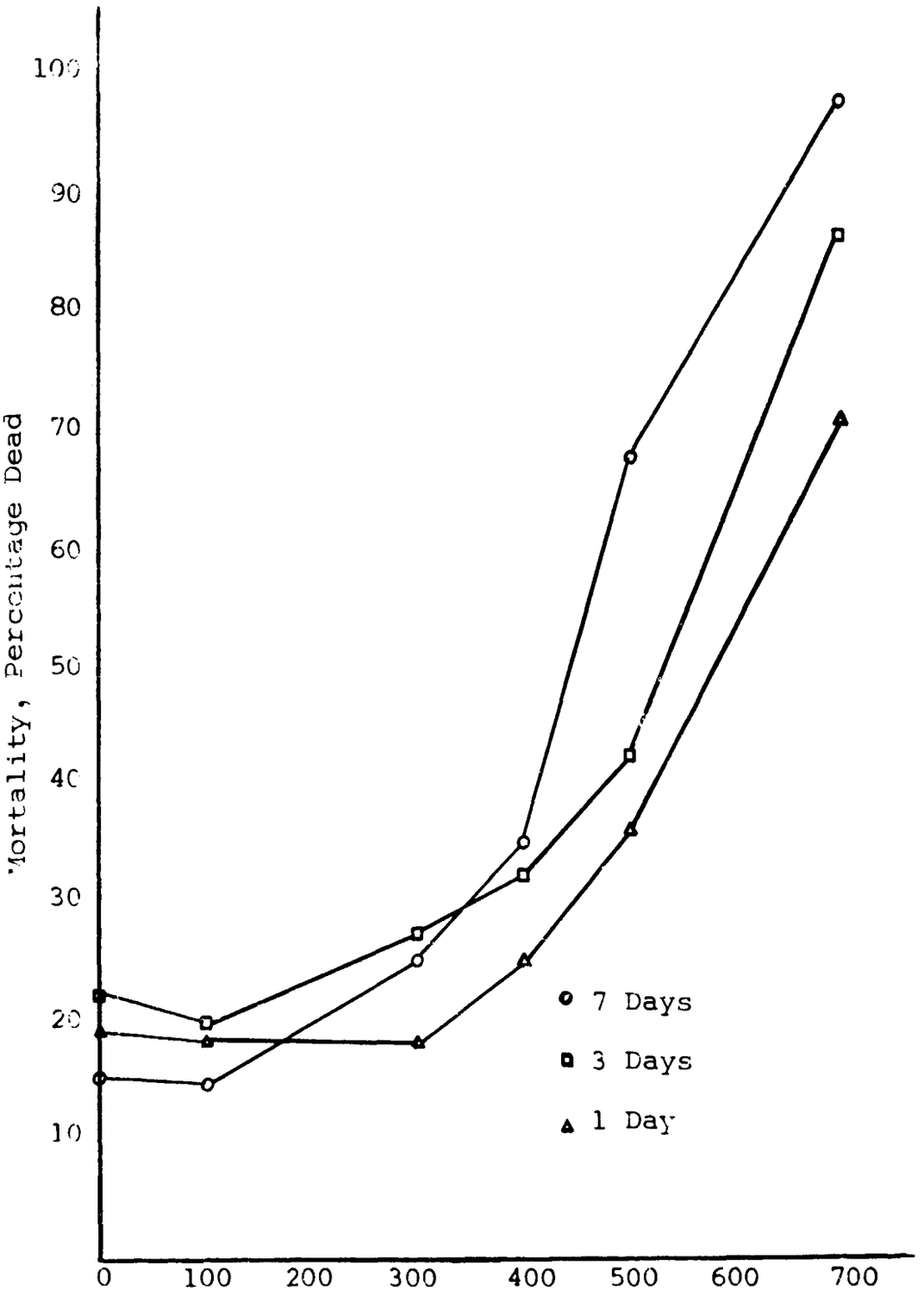

Figure 5

EFFECT OF TIME INTERVAL BETWEEN RADIATION AND BACTERIAL CHALLENGE WITH AVIRULENT B. ANTHRACIS, STRAIN 32R

significantly lethal numbers of organisms are included, significant increases in susceptibility to infection in populations exposed to radiation doses of less than 100 rads can be expected.

The results of the experiments in which the VIb-189 virulent strain of B. anthracis was utilized are presented in Tables 11, 12, and 13. A pronounced combined effect occurred, especially at 500 rads. These results agree very well with the results obtained using strain 32S as the challenging organism.

Experiments currently being carried out are utilizing Venezuelan equine encephalomyelitis (VEE) virus as the infectious organism, and in future work the infectious organism will be Coxiella burnetii. The effects of chronic and periodic exposures to radiation before infectious challenge and the effects of irradiation on the effectiveness of immunization with these organisms will be investigated.

Table 11

MORTALITY OF MICE EXPOSED TO RADIATION
AND INOCULATED 3 DAYS LATER
WITH VIRULENT B. ANTHRACIS, STRAIN V1b-189

Radiation Dose, rads	Mortality, deaths/total Number of Organisms 0	3	7	65
0	0/40	8/40	13/40	21/40
100	0/40	7/40	16/40	24/40
300	4/40	8/40	16/40	27/40
500	15/40	28/40	30/40	38/40

Table 12

SURVIVAL OF MICE EXPOSED TO RADIATION
AND INOCULATED 3 DAYS LATER
WITH VIRULENT B. ANTHRACIS, STRAIN V1b-189

Radiation Dose, rads	Cumulative Percent Survival Number of Organisms 0 Exp	3 Exp	3 Calc	7 Exp	7 Calc	65 Exp	65 Calc
0	100	80		70		47	
100	100	82	80	60	70	40	47
300	90	80	72	60	63	32	42
500	62	30	50	25	43	5	29

Table 13

PERCENTAGE OF EXCESS DEATHS
RESULTING FROM EXPOSURE OF MICE TO RADIATION
AND INOCULATION WITH B. ANTHRACIS STRAIN V1b-189

Dose, Rads	Percentage Number of Organisms 3	7	65
100	-3	14	16
300	-7	4	24
500	40	41	83

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13 ABSTRACT

Exposure of Swiss albino mice to various levels of Co^{60} gamma radiation and subsequently to various levels of infection with virulent and avirulent strains of Bacillus anthracis indicates that a significant pronounced combined effect is present. At low levels of radiation, bacterial challenges near the LD_{50} are necessary to demonstrate the combined effect while at high levels of radiation a few organisms produce a pronounced combined effect. The time interval between radiation and infectious challenge significantly affects the degree of interaction with a 7 day interval resulting in higher mortalities than either a 1 or 3 day interval.

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14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Combined effects, radiation and bacterial infection. Gamma radiation, B. anthracis infection.						

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